bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Immuno-fluorescent (IF) staining

Minyan Zheng Veronica F Hinman

Updated date: Sep 19, 2022

An abbreviated version of this protocol was published in eLIFE in Jan 2022

Regeneration of the larval sea star nervous system by wounding induced respecification to the Sox2 lineage

DOI: 10.7554/eLife.72983

Detailed protocol

1. Fix the samples in 4% PFA in 1x PBS, pH 7.4 (see notes below)
2. Wash 1x 15 min in PBS to remove PFA
3. Postfix in 100% cold Methanol for 10 min at -20°C
4. Step into 0.1% triton x100 / PBS
5. Permealize membranes in 0.5% triton X100 / PBS for 30 min at RT
6. To quench autofluorescence incubate in 0.1M glycine in 0.1% triton X100 / PBS for 30 min at RT
7. Wash 3 x15 min in 0.1% triton X100 / PBS
8. Incubate the samples with Blocking Solution (3% BSA in 0.1% triton X 100 / PBS) for 1h at RT
9. Prepare the 1st antibody
10. Vortex and centrifuge Ab stocks in their original vials at 14000 rcf for 5 min.

Dilute the 1st antibody in Blocking Solution (if applicable).

1. Apply the 1st antibodies overnight at 4°C.
2. Wash 6 x15 min in Blocking Solution at RT
3. Prepare the 2nd antibody:

Vortex and centrifuge Ab stocks in their original vials at 14000 rcf for 5 min
Dilute the 2nd antibody in Blocking Solution

1. Apply the 2nd antibody for 1h at RT
2. Wash 3 x 15 min in Blocking Solution
3. Wash 3 x 15 min in 0.1% triton X100 / PBS
4. Nuclear staining with DAPI – 1:10000 in PBS for 30 min at RT
5. Wash 3 x 5 min in PBS
6. Remove the excess of PBS and add the antifading medium.
7. Image!

Notes:

1. The fixative should be freshly prepared before use. Can be stored for 1 week at 4ºC.
Fixation time can vary from overnight incubation at +4°C to 15 min – 2h at RT.
PFA: Paraformaldehyde

2. 3-5% normal goat serum solution in 0.1 % triton X 100 / PBS can be use once at step 8 to block non-specific binding from the goat 2nd antibody.

Antifading medium recipe:
DABCO (Aldrich, D2522) – 2.5%
Glycerol – 25%
in 0.2 M Tris-Hcl , pH 8.5

Notes specific to request: Reduce background by 1. Proper fixation - over fixation can lead to background. 2. Correct dilution of antibody. In the first experiment prepare a titration of antibody concentrations to identify the optimum signal to background. 3. Carefully rotate samples while washing after antibody treatment and ensure good wash exchanges (i.e. wash in 1 ml and remove all but 50 to 100ul of solution after each wash)

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Zheng, M. and F Hinman, V. (2022). Immuno-fluorescent (IF) staining. Bio-protocol Preprint. bio-protocol.org/prep1953.
2. Zheng, M., Zueva, O. and Hinman, V. F.(2022). Regeneration of the larval sea star nervous system by wounding induced respecification to the Sox2 lineage. eLIFE. DOI: 10.7554/eLife.72983